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SUPPLEMENTARY MATERIALS

Nutraceutical properties and phytochemical characterization of wild Serbian fruits

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Antioxidant activity assays

DPPH˙ Scavenging Assay. DPPH radical scavenging activity was tested using a slightly modified method published by [27]. 500 µL of sample extracts were mixed with DPPH˙ reagent solution to a 1 mL final volume. After the incubation (20 min at room temperature), absorbance was recorded at 517 nm. To calculate DPPH radical scavenging activity (%) the following equation was used:

DPPH˙ scavenging (%) $=\{(A_{517\ control} - A_{517\ sample})/ A_{517\ control}\}\times 100$

Trolox C standard calibration curve (100-600 µM) was constructed and the results were expressed as mmol of Trolox equivalents per Kg of frozen sample (mmol TE/Kg FW).

Fe^{2+} chelating capacity (FCC) assay. To define metal chelating ability a previously reported procedure was followed [28]. The final reaction mixture contained 200 µL of sample, 20 µL of 2 mM $FeCl_2 \cdot 4H_2O$, 200 µL of 5 mM ferrozine solution and 1080 µL of distilled water. After the incubation (10 min at room temperature), the absorbance was recorded at 562 nm. To calculate FCC % the following equation was used:

Fe^{2+} chelating capacity (%) $=\{(A_{562\ control} - A_{562\ sample})/ A_{562\ control}\}\times 100$

Fe^{3+}reducing capacity (FRC) assay. Reducing capacity was evaluated by the modified method of [29]. Phosphate buffer (pH 6.6, 500 µL 0.2 M) and potassium ferricyanide (III) (400 µL, 10 mg mL^{-1}) were mixed with samples (200 µL aliquots of the dilutions in methanol). After the incubation of mixtures for 20 min at 50 °C in a water bath,

trichloroacetic acid (500 µL, 10%, v/v) was added and samples were centrifuged (10 min at 2000 × g). Aliquots of supernatants (500 µL) were then mixed with distilled water (500 µL) and $FeCl_3$ (100 µL, 1 mg/mL), left for 10 min at room temperature before the measurement of the absorbance at 700 nm. Reducing capacities of the extracts were expressed as absorbance units at 700 nm.

Nitric oxide (NO) scavenging activity. NO scavenging activity test was performed according the method of [30]. 25µL of diluted extracts (1:100, 1:200, and 1:500) and 75µL of freshly prepared sodium nitroprusside 5mM in phosphate buffer saline solution (pH 7.4) were incubated at 25 °C for 150 min, and then added of 75µL Griess reagent. The absorbance at 546 nm was recorded after 10 min of incubation. Negative control sample without extract, but with the equivalent amount of reagents was prepared. The results were expressed as percentage of scavenged NO with respect to the negative control. Ascorbic acid (0.06-1 mg/mL) was used as a positive control.

Anti-tyrosinase activity (Tyr). The tyrosinase inhibitory activity test was performed by mixing 50µL of extract samples with 0.9 mL of phosphate buffer solution (0.1M, pH 6.8) and 0.5 mL of L-DOPA (0.03% in phosphate buffer 0.1M, pH 6.8). After incubating the mixture for 10 min at 37 °C, 50µL of tyrosinase solution (1000 U/mL) was added, and the mixture was again incubated for 5 min at 37 °C. The absorbance at 475 nm was recorded [31]. The anti-tyrosinase activity was calculated as follows:

Anti-tyrosinase activity (%) $=\{(A_{475\text{ reaction mixture without sample}} - A_{475\text{ reaction mixture without sample and tyrosinase}}) - (A_{475\text{ reaction mixture}} - A_{475\text{ reaction mixture without tyrosinase}})/ (A_{475\text{ reaction mixture without sample}} - A_{475\text{ reaction mixture without sample and tyrosinase}})\}\times 100$

Ascorbic acid (0.5-2.5 mg/mL) was used as a positive control.

Analysis of polyphenols

Polyphenols were separated, quantified and identified using UHPLC system (Dionex Ultimate 3000, ThermoFisher Scientific, Bremen, Germany) equipped with a diode array detector (DAD) and triple-quadrupole mass spectrometer. Chromatographic separations and quantitative analysis of phenolic compounds were achieved following the optimized previously reported method [26].

The chromatograms were recorded at 254 nm. The injection volume and the flow rate were 5 µL and 0.4 mL/min, respectively.

Quantification was done using available phenolic standards. A 1 mg/mL stock methanolic solution of a mixture of all standards was prepared and dilutions were prepared in methanol to obtain working solutions at 0.025, 0.050, 0.100, 0.250, 0.500, 0.750, and 1.0 mg/mL. Retention times (t_R, min), mass of parent ions (*m/z*) and product ions (*m/z*),

correlation coefficients, limits of detection (LOD) and quantification (LOQ) of the phenolics quantified in fruit samples are presented in supplementary file (Supplementary Table S1).

Table S1. Mean expected retention time (t_R, min), mass of parent ion (*m/z*), masses of product ions (*m/z*), correlation coefficient, limit of detection (LOD), limit of quantification (LOQ) of the quantified polyphenols.

No	Compound name	Retention time , min	Parent ion, $[M-H]^-$ (*m/z*)	Product ions, MS^2 fragments $[M-H]^-$ (*m/z*)	R^2	LOD (mg/L)	LOQ (mg/L)
1	**Arbutin**	1.77	271.069	108.28; 161.22	0.9922	0.16	0.55
2	**Gallic acid**	2.22	169.032	79.11; 125.04	0.9965	0.15	0.50
3	**Protocatechuic acid**	4.10	153.003	108.07; 109.10	0.9976	0.10	0.34
4	**Aesculin**	4.86	339.080	133.09; 177.06	0.9977	0.09	0.31
5	***p*-Hydroxybenzoic acid**	5.20	137.057	93.19; 108.33	0.9953	0.16	0.54
6	**5-*O*-Caffeoylquinic acid**	5.31	353.103	191.28	0.9992	0.06	0.21
7	**Caffeic acid**	5.64	179.004	134.08; 135.11	0.9917	0.18	0.61
8	**Vanillic acid**	5.65	167.034	108.10; 153.06	0.9910	0.15	0.50
9	**Syringic acid**	5.80	197.040	153.02; 182.02	0.9954	0.14	0.48
10	***p*-Coumaric acid**	6.39	163.031	93.12; 119.09	0.9971	0.11	0.37
11	**Ellagic acid**	6.57	300.980	228.95; 284.00	0.9937	0.11	0.37
12	**Ferulic acid**	6.75	193.057	134.01; 197.02	0.9923	0.18	0.60
13	**Catechin**	5.21	289.084	203.00; 245.03	0.9943	0.16	0.53
14	**Rutin**	6.23	609.197	299.98; 301.20	0.9982	0.10	0.33
15	**Quercetin 3-*O*-galactoside**	6.40	463.002	271.01; 300.02	0.9908	0.18	0.62
16	**Apigenin 7-*O*-apioglucoside**	6.73	563.056	267.98; 269.01	0.9910	0.17	0.56
17	**Naringin**	6.75	579.241	151.42; 217.36	0.9945	0.16	0.53
18	**Kaempferol 3-*O*-glucoside**	6.83	447.008	255.03; 284.03	0.9922	0.05	0.16
19	**Phlorizin**	7.17	435.169	167.16; 273.16	0.9901	0.21	0.70
20	**Luteolin**	8.17	285.035	133.05; 150.95	0.9927	0.22	0.74
21	**Apigenin**	8.91	269.032	117.24; 151.10	0.9920	0.19	0.63
22	**Naringenin**	8.93	271.036	151.07; 119.10	0.9967	0.14	0.46
23	**Kaempferol**	9.02	285.074	211.23; 227.16	0.9913	0.17	0.56